EXHIBIT K

Materials Characterization of Explanted Polypropylene Hernia Meshes

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Abstract: Hernia repair with prosthetic mesh significantly decreases the rate of recurrence compared with traditional, primary suture repair by reducing the tension on the edges of the wound. However, there are several complications associated with the use of mesh that may be due to the chronic inflammatory reaction to the mesh or a loss of compliance after degradation of the material. Mesh contraction and migration can also occur, sometimes resulting in a recurrent hernia. Based on the chemical structure of the polypropylene mesh material and the physiological conditions to which it is subjected, it is possible that oxidation is responsible for these changes in material properties. Oxidation would result in surface cracking, decreased melting temperature, loss of mass, and reduced compliance of the material. The objective of this study was to identify physiochemical changes in the surface and bulk properties of explanted polypropylene hernia meshes compared to pristine polypropylene mesh materials. Several characterization techniques were utilized, including scanning electron microscopy, differential scanning calorimetry, thermogravimetric analysis, and compliance testing. Overall, the results supported our hypothesis that oxidation is involved with the degradation of polypropylene hernia mesh materials. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 83B: 44–49, 2007

Keywords: differential scanning calorimetry (DSC); hernia meshes; oxidation; polypropylene; scanning electron microscopy (SEM); thermogravimetric analysis (TGA)

INTRODUCTION

Over 20 million hernia repairs are performed worldwide each year using a variety of surgical techniques.[1] Historically, hernia repairs were accomplished by approximating the edges of the abdominal fascial defect using wire, and later polypropylene permanent sutures.[2] Unfortunately, this type of repair places a great deal of tension on the wound, leading to tissue ischemia, wound dehiscence, and a recurrence rate of up to 63%.[2] During the past 50 years, several techniques for both open and laparoscopic hernia repair have been developed, including underlay (intraperitoneal and preperitoneal), onlay, and inlay placement of a mesh material to repair the defect. These techniques have been shown to reduce recurrence rates to 32% or less for open repair[3] and less than 5% for laparoscopic repair.[4]

Despite this tremendous decrease in the hernia recurrence rate, which prosthetic implants have made possible, these materials can be problematic. In 2002, Kumar et al. reported that approximately 30% of patients experience pain or discomfort following inguinal hernia repair, and more than half of those patients are unable to participate in daily, physical or athletic activities due to the severity of the pain.[5] There are many potential sources of chronic pain, including stiffening of the abdominal wall due to an intense inflammatory reaction to the mesh material. In addition, nerve damage may result from entrapment in this scar tissue or from the mesh fixation method utilized in the surgical procedure. It is possible that an ongoing inflammatory response to the permanent implant is responsible for these complications.

Despite the potential for improvement in recurrence rates and wound complications, a laparoscopic hernia repair still involves implantation of a foreign body, and mesh fixation causes injury to the surrounding tissue. This activates phagocytic cells such as neutrophils, monocytes, and macrophages to respond to the injury, where their primary roles are to degrade debris and foreign materials prior to wound healing, as well as to recruit more inflammatory cells to the site. To accomplish this, these cells metabolize oxygen and secrete lysosomes containing radicals such as superoxide anions, as well as very strong oxidants such as hydrogen peroxide and

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Figure 1. Kugel Composix hernia mesh immediately after explantation.

hypochlorous acid.[6] In the case of a large, permanent implant such as a hernia mesh, the material will never be completely degraded and removed from the body. This results in continuous activation of phagocytic cells and generation of oxidants. Histological studies have documented this prolonged inflammatory reaction over the lifetime of the implant. In a study of several types of explanted materials, including polypropylene, expanded polytetrafluoroethylene, and polyester terephthalate, Klinge et al. reported that cells such as macrophages, polymorphonuclear leukocytes, and lymphocytes are present at the tissue-mesh interface even up to 8 years after the surgery.[7] Specialized cells called foreign-body giant cells begin to populate the surface of the mesh, and can lead to granuloma formation.[8] This prolonged inflammatory response is thought to cause fibrosis and a rigid scar plate to form around the mesh material, particularly in the case of polypropylene meshes, leading to chronic pain and reduced mobility.[8,9]

As a result of this chronic inflammatory response, the mesh material is exposed to a continuous bath of oxidants. Aliphatic hydrocarbons such as polypropylene are known to be highly susceptible to oxidative attack. These materials contain functional groups that are easily cleaved, which then produces a resonance stabilized free radical.[6] Continuous exposure of polypropylene to these oxidants may lead to chain scission, production of free radicals, and overall degradation of the material, both physically and chemically.[10–13] This degradation is evidenced by fissures, microcracks with a build-up of hydroxyl and carbonyl groups on the surface of the material, changes in thermal properties such as decreased glass transition and melting temperatures, weight loss, and changes in mechanical properties such as embrittlement and reduced compliance.[10–13]

Because of the susceptibility of polypropylene to oxidation and the evidence of embrittlement and reduced compliance of the material *in vivo*, it is our hypothesis that oxidation is responsible for the some of the complications associated with polypropylene hernia repair materials. We expect that the physiochemical changes that are indicative of oxidation will be apparent after characterization of explanted mesh materials via scanning electron microscopy (SEM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and compliance testing.

MATERIALS AND METHODS

Explanted Hernia Meshes

Polypropylene hernia repair materials were removed from patients requiring revision surgery due to chronic pain, recurrence, infection, adhesions, or other complications. An example of an explanted mesh is shown in Figure 1, and the contraction and embrittlement of the material are evident. The study was approved by the University of Missouri-Columbia's IRB committee, and each explant was given a sequential specimen number unrelated to any identifiable patient data. All 14 samples included in this study were the polypropylene components from polypropylene/expanded polytetrafluoroethylene composite hernia mesh such as Composix E/X or Kugel Composix (C.R. Bard, Cranston, RI), shown in Figure 2.

Tissue Removal

After explantation, the meshes were immersed in a 10% v/v formalin solution and stored at room temperature. Prior to testing, any adherent tissue was removed from the meshes by soaking in a sodium hypochlorite solution for 2 h at 37°C (6–14% active chlorine, Sigma Aldrich, St. Louis, MO). Each mesh was then rinsed several times with distilled water to remove any residual sodium hypochlorite solution and allowed to dry overnight. The explanted mesh shown earlier

Figure 2. Pristine Kugel Composix hernia mesh.

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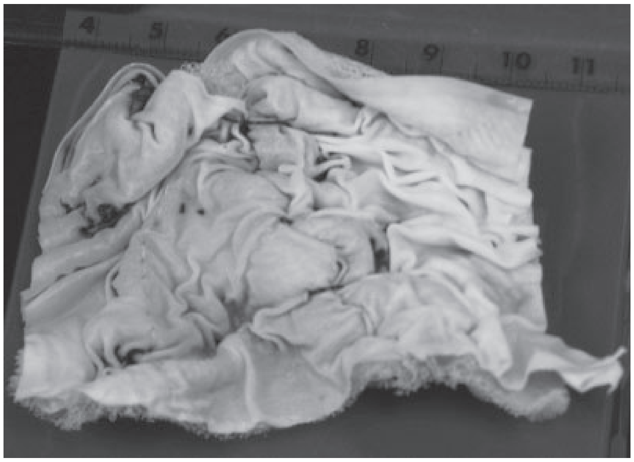

Figure 3. Explanted Kugel Composix hernia mesh after tissue removal.

is now shown in Figure 3 after the tissue has been removed. The mesh remains contracted, suggesting a permanent deformation of the material while *in vivo*. Pristine samples of a Composix E/X mesh (C.R. Bard, Cranston, RI) were also subjected to the same cleaning process and used in all subsequent tests for comparison with explanted samples.

Scanning Electron Microscopy

Micrographs of each explanted mesh, as well as those from a pristine Composix E/X mesh, were obtained using a Hitachi S-4700 Scanning Electron Microscope with a secondary electron detector operated at an accelerating voltage of 5 keV. Prior to imaging, all samples were mounted and sputter-coated with platinum for 120 s at 20 mA (Emitech K575X Peltier Cooled Sputter Coater, Emitech Products, Houston, TX).

Differential Scanning Calorimetry

DSC was performed in order to identify changes in melting temperature and heat of fusion. Mesh samples having a mass of 1–3 mg were hermetically sealed in aluminum pans, and an empty pan was used as a reference. A PyrisTM1 Differential Scanning Calorimeter with TAC 7/PC Thermal Analysis Controller (Perkin-Elmer, Norwalk, CT) was programmed to heat the sample from 50 to 400°C at 10°C/min under the flow of nitrogen and record changes in heat flow as a function of temperature.

Thermogravimetric Analysis

TGA was carried out in order to measure chemical changes in the explanted mesh materials such as decomposition of the material. This was quantified as the percent of weight lost during the thermal event. Samples of both explanted and pristine meshes were cut to fit within the sample pan of the TGA (approximately 5 mm × 5 mm). A Q Series Thermogravimetric Analyzer, TGAQ50 (TA Instruments, New Castle, DE), was programmed to heat the sample from ambient temperature to 600°C at 10°C/min under the flow of nitrogen and record the weight lost as a function of temperature.

Compliance Test

Prior to performing compliance testing, each explanted mesh, as well as a pristine Composix E/X, were cut into three squares (6.45 cm^2 each) and soaked overnight in 1× phosphate-buffered saline (pH 7.4) at 37°C to allow for equilibration at physiological conditions. A Texture Analyzer, TA.XT2, with XTRA Dimension software (Texture Technologies, Scarsdale, NY) was then programmed to simulate the physiological conditions of bending at the waist or abdominal crunch exercises. This was accomplished by using a rounded blade to bend the mesh in half and push it through a 2.92 cm^2 slot at a rate of 0.2 mm/s. The total work in Joules was recorded and analyzed as a measure of the compliance of the material.

Statistical Analyses

All statistical analyses were carried out using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). A one-way analysis of variance with a 95% confidence interval and Dunnett's post-test was used to determine whether the differences in the means of the explanted materials versus a pristine sample were significant ($n = 3$, $p < 0.05$).

RESULTS

Scanning Electron Microscopy

As shown in Figure 4, SEM micrographs of the polypropylene component of a pristine Composix E/X mesh revealed smooth fibers without any evidence of surface degradation

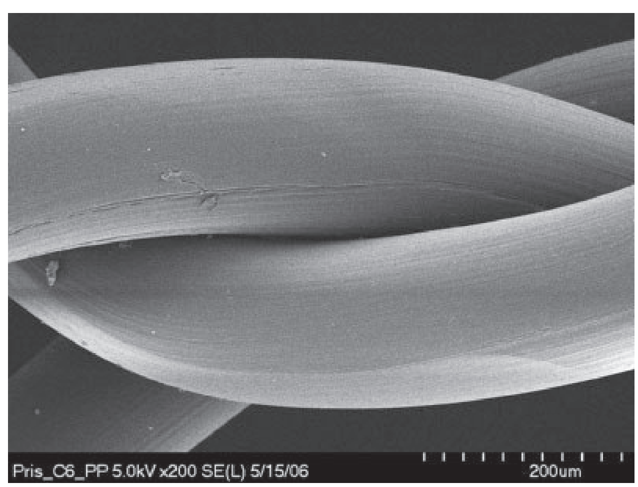

Figure 4. SEM of the polypropylene component of a pristine Composix E/X mesh.

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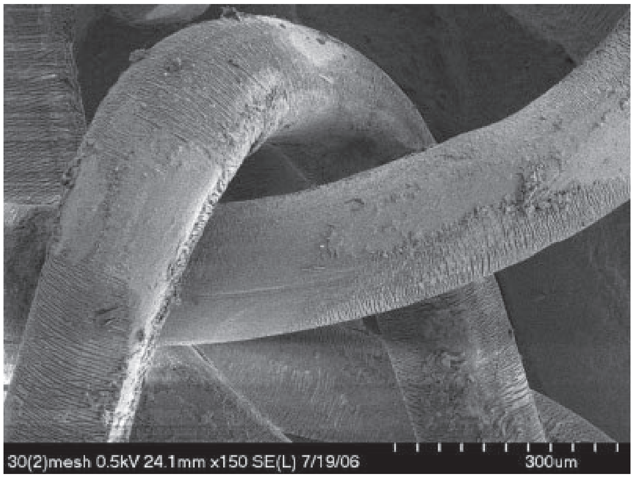

Figure 5. SEM of an explanted polypropylene mesh with transverse cracks.

such as cracks or fissures. Conversely, as shown in Figures 5–7, the explanted polypropylene specimens appeared badly degraded, including cracks, fissures, and increased surface roughness. Micrographs of 79% of all explanted specimens exhibited cracks in the transverse or longitudinal direction, 57% showed what appeared as a peeling of the fiber surface, and 64% displayed a rough, blistered surface. Overall, 85% of the explanted specimens possessed at least two or more of these types of surface structures.

Differential Scanning Calorimetry

Figure 8 depicts the results of DSC for two explanted specimens versus the pristine polypropylene specimen. Although this graph depicts only two explanted specimens, it is representative of the results obtained for all 14 explants. All explants displayed a lower melting temperature of approximately 163°C on average as compared to 166°C for the pristine, with five of the 14 explants having a p-value < 0.05. Half of the explants exhibited broader melting peaks, and the majority possessed a smaller area under the curve.

Thermogravimetric Analysis

After TGA, each thermogram was integrated to obtain the area under the curve. This provided a quantitative analysis of the entire thermal event of each explant, rather than just an analysis of the conditions at the peak temperature. Figure 9 depicts the resulting thermograms for two explanted specimens versus the pristine polypropylene specimen. All explanted specimens exhibited a shift in the peak temperature from 458°C for pristine materials to a higher temperature of approximately 465°C on average for the explanted meshes. The majority of the explants had a p-value < 0.05, and nearly all displayed a smaller mean area under the curve of 9.67% versus 12.04% for pristine materials.

Compliance Test

The mean value for the total work required to fold each mesh in half and push it through the slot is shown in Figure 10. Nearly all explanted materials required more work than the pristine Composix E/X mesh, with values ranging from 4 times the work required for a pristine mesh to nearly 30 times this value, indicating an overall decrease in the compliance of these materials.

DISCUSSION

The objective of this study was to determine whether oxidation plays a role in the degradation of polypropylene hernia materials while *in vivo*. Thus, physiochemical analysis was performed on 14 explanted specimens as well as pristine specimens. The results from SEM, DSC, TGA, and compliance testing provided strong support that oxidative degradation was occurring *in vivo*.

The SEM micrographs displayed images of materials that were vastly different in topology than the pristine materials. The micrographs of explanted polypropylene materials exhibited cracks, surface roughness, and peeling indicative of surface degradation,[6,10,12] while the pristine materials appeared smooth.

DSC results displayed melting temperatures and heat of fusion in explanted materials that were lower than the pristine values. The melting peak of the explanted materials was also broader; most likely due to increased polydispersity of the material. The majority of the explanted polypropylene mesh samples displayed a change from the pristine for all three of these effects, indicating that oxidation of the material may have occurred while *in vivo*; however, not all of these effects were considered statistically significant. The lack of statistical significance for all the samples may be due to the shorter implantation times experienced by some of the explants.

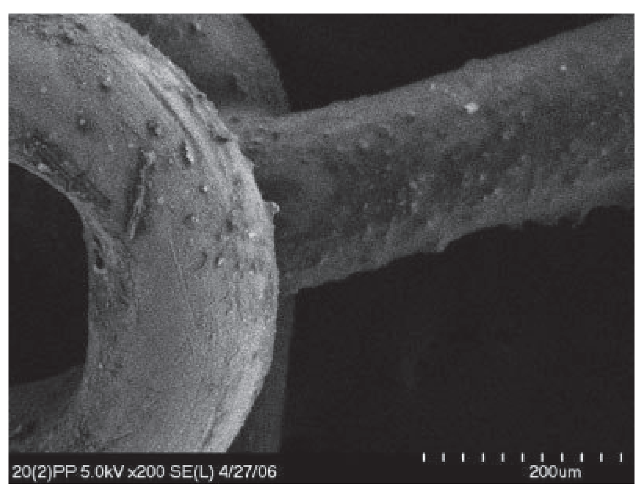

Figure 6. SEM of an explanted polypropylene mesh with blisters on the fibers.

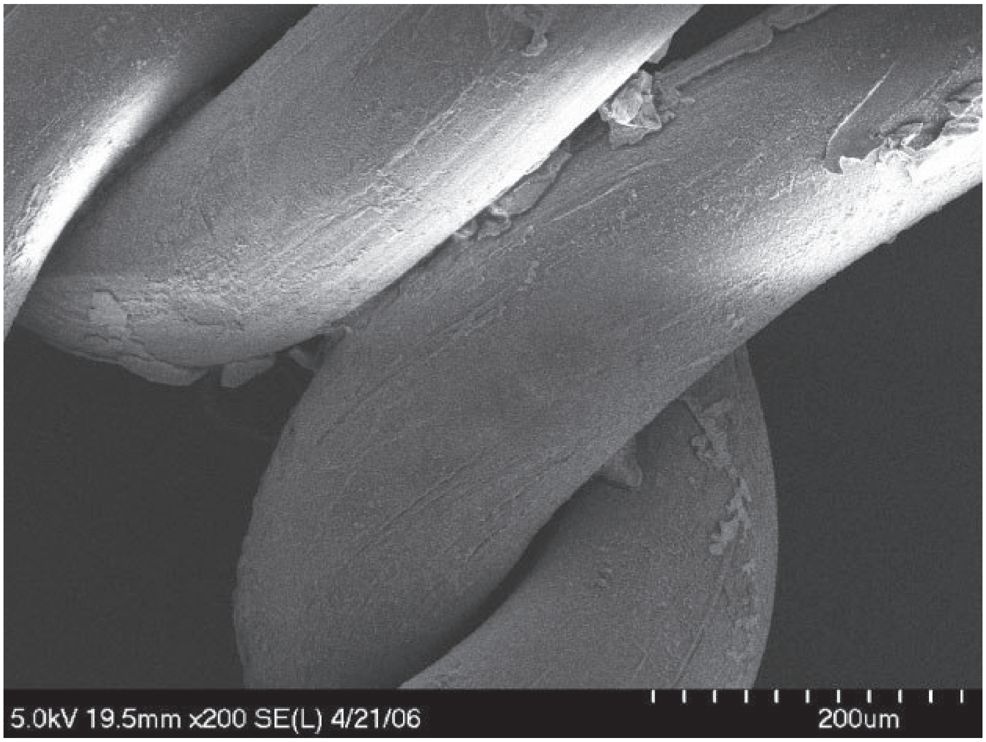

Figure 7. SEM of an explanted polypropylene mesh with "peeling" fibers.

Oxidized materials are expected to undergo some degree of weight loss as the material is degraded by the body.[10,12] Thus oxidized materials should have less weight available to be lost during TGA. Weight loss during TGA is reflected in the area under the curve, and so oxidized materials should display less area under the curve. As expected, the majority of the explanted polypropylene specimens showed significantly less weight loss during TGA as compared to the pristine Composix E/X specimen, further strengthening the argument that these materials underwent oxidation *in vivo*.

The results of the compliance test on explanted polypropylene materials indicate reduced compliance in all but one explant, which is evidence of oxidation[11,13] as well as a potential explanation for decreased abdominal wall function and chronic pain. Welty et al. examined patients using 3D stereography to measure the stiffness of the abdominal wall following hernia repair with a polypropylene mesh material. The results of this study indicated that patients complained of paresthesia 4–58% of the time, depending on the "*weight*" (g/m^2) of the polypropylene material and had

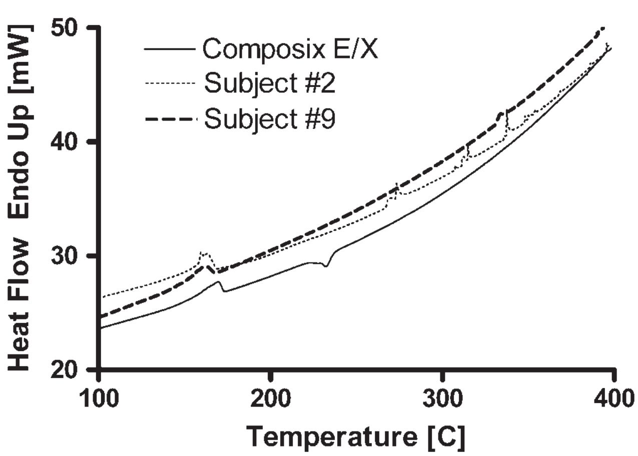

Figure 8. DSC thermograms of explanted and pristine specimens.

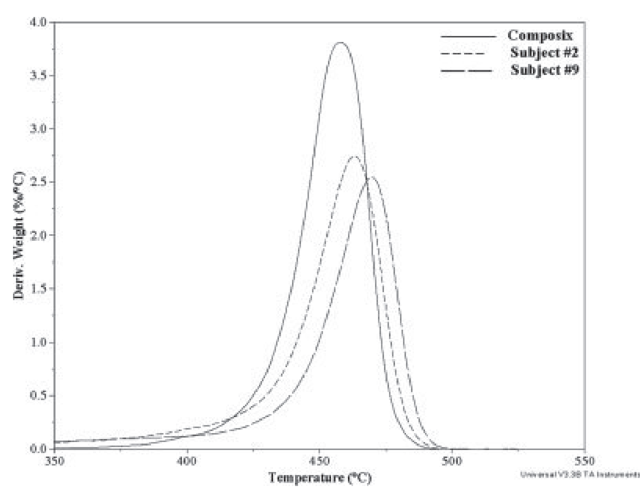

Figure 9. TGA thermograms of explanted and pristine specimens.

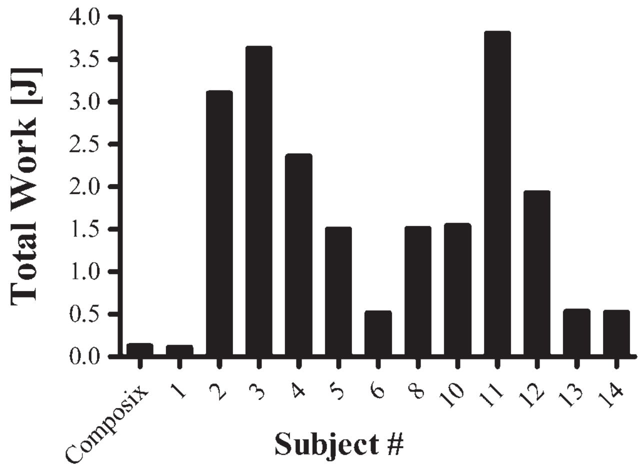

Figure 10. Total work required for compliance testing of explanted and pristine specimens.

difficulty performing daily tasks up to 17% of the time. Three-dimensional stereography of three types of polypropylene revealed decreased abdominal wall curvature and decreased height. These findings correspond to increased abdominal wall stiffness and patient complaints of pain and restricted mobility.[8]

During the implantation period, the surface of the explanted materials stimulated the foreign body response that, in turn, produced oxidants such as hydrogen peroxide and hypochlorous acid. Polypropylene is susceptible to oxidation due to its chemical structure. During oxidation of polypropylene, the C—H bonds along the polymer backbone are broken, leading to bulk degradation of the polymer.[10,12] While the analysis of the physiochemical factors of the explanted and pristine specimens provided evidence of oxidation, there are many factors that will affect the severity of oxidation. Since the explants were removed from the patients due to complications of the mesh, the explanted materials have a variety of implantation times ranging from months to years. The severity of the oxidation of the polypropylene material is likely affected by the implantation time, as well as other unique, patient factors such as age, history of smoking, body mass index (BMI), pulmonary disease, diabetes, and potential genetic variation, including variable inflammation and collagen defects. Because all 14 explanted specimens had different implantation times and different patient histories, not all of the findings were statistically significant. Future studies will focus on these factors that may contribute to the rate of degradation, and currently, a manuscript is in progress that correlates many of these factors to the rate of material degradation. In addition, other tests will be performed, such as chemical analyses using Fourier transform infrared spectroscopy (FTIR). FTIR would provide information about the chemical species present on the surface of the materials, further strengthening the evidence of oxidation. Future tests may also include molecular weight determination, which was not performed here due to the difficulty in determining the molecular weight of a 2 component (polypropylene and expanded polytetrafluoroethylene) composite hernia mesh.

CONCLUSION

An investigative study was performed to identify physiochemical changes in the surface and bulk properties of explanted polypropylene hernia meshes compared to pristine samples. Our results supported our hypothesis and indicated that the explanted polypropylene meshes did undergo degradation while *in vivo*, most likely due to oxidation.

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